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CORRELATION OF THE VOCAL FOLD VIBRATORY PATTERN
TO THE POST-OPERATIVE SURGICAL WOUND IN THE PORCINE MODEL

FINAL REPORT

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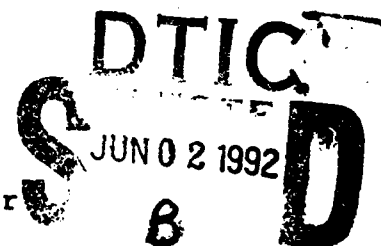
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13. ABSTRACT (Maximum 200 words) Surgical injury to the human vocal folds causing changes in vibration, cannot be studied in a well-controlled fashion due to ethical considerations. Therefore, miniswine served as an animal model to study the effect on vibratory function after surgical injury to the vocal folds. Twelve miniswine were operated upon in staged procedures. Initially, the right true vocal folds of the miniswine were injured by creating progressively deeper biopsies from the epithelial cover through the vocalis muscle. The left true vocal fold served as a control. After healing, a second procedure was conducted to study the vibratory function utilizing stroboscopy and electroglottography during artificial phonation. These results were compared to the histological findings which showed that injury at the junction of the lamina propria and vocalis muscle caused significant vibratory dysfunction. Because the miniswine and human larynges are similar, the otolaryngologist can expect unsatisfactory phonation once injury occurs at this critical junction.				
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Introduction

Research in vocal physiology has been conducted using living human larynges, excised cadaver larynges, animal models, mechanical and computer systems¹⁻³. Most researchers have studied the larynx as a normal structure. A few studies have attempted to characterize altered vocal fold vibration due to unilateral nerve paralysis^{10,11} and asymmetric vocal fold tension^{12,13}. The effect of surgery on the vocal fold anatomy and physiology has received limited study. Most studies have involved retrospective clinical reports of patient populations in which vocal folds have been operated upon for benign or malignant lesions^{14,15}. Controlled, prospective studies are uncommon. Animal models are a more acceptable and ethical approach to this problem. The present investigation was designed to study the effect on the vocal fold function by creating progressively deeper surgical wounds in the vocal fold using an in vivo porcine model. The objective was to demonstrate and to describe the histological correlation between the vibratory dysfunction and the scar formation at different functional layers within the vocal folds. To do this, videostroboscopy (VS) and electroglottography (EGG) were used to record glottal function during artificially induced phonation.

Scar formation after surgical injury to the vocal folds is considered the cause for vocal fold dysfunction. The scar is demonstrated by stroboscopy as a non-vibratory or stiff segment of

the vibrating vocal fold. This observation is based on studies conducted by Ohkubo¹³ who described scar formation after laser surgery. Hirano¹⁴ supported these findings by comparing vocal fold function in patients undergoing laser surgery for T₁ glottic carcinoma with that of patients receiving radiation treatment for similar lesions. The depth of the tissue removed was correlated with vocal fold dysfunction. Histological confirmation was not reported. Studies by Leonard et al.^{21,22} examined the microscopic changes due to repeated mucosal stripping of the vocal folds in cats. Repeated stripping caused progressive increase in the amount of scar tissue and decrease in the numbers of nerve endings within the mucosal folds. The loss of nerve endings was hypothesized as the cause of vocal fold dysfunction due to decreased proprioception.

A clearer understanding of vocal fold dysfunction due to surgical injury requires study in an animal model system with vocal folds that are similar to those of the human vocal folds. Animal larynges are easier to obtain and the precise timing of study can be controlled. Theories of vocal fold function can be tested and the lessons learned in the laboratory help the laryngologist provide a better quality of care for the patient. Miniswine were selected for this study based upon the findings of Kurita²³ who conducted comparative studies of the layered structure of the vocal fold in animals. Historically, the dog has been widely used as a



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study animal, but dog vocal folds differ significantly from human vocal folds. The mechanical properties of the vocal fold of the miniswine, are however, closest to those of the human. The key differences and similarities are summarized in Table I.

Theories of vocal fold vibratory physiology have been reviewed in a concise manner by Kitzing²⁴. Hirano has proposed the body-cover complex²⁵ and traveling mucosal wave²⁶ theories that are important to this study. His description of the human vocal fold consists of three functional layers: a cover consisting of the epithelium and the loose outer layer of the lamina propria; a transition layer consisting of the middle and deep layers of the lamina propria (the conus elasticus); and, a muscular body consisting of the vocalis muscle. The body-cover theory of vocal fold vibration describes phonation as a product of the interaction of the mucosal, transitional, and muscle layers. The miniswine differs because the conus elasticus is not a distinct layer. Despite this, reference will be made throughout the article to the body-cover complex. For the miniswine, the cover consists of the epithelium and the superficial layers of the lamina propria; the body consists of the deeper layers of the lamina propria and the vocalis muscle.

High speed films and stroboscopy have demonstrated the transverse movement of the body and the vertical traveling wave

movement of the cover²⁷. The medial vibrating edge of the vocal fold has both upper and lower functional margins. With each cycle vibration, a glottal puff of air is formed as the lower margin opens first, followed by opening of the upper margin and release of the puff. This fluid-like movement is defined as a traveling mucosal wave²⁸. Stroboscopic examination can differentiate normal from pathological vocal folds on the basis of the movement of this wave. The miniswine model system reproduces this traveling mucosal wave with artificial phonation in order to study changes in vocal fold vibration.

The purpose of this paper is to define the depth of surgical excision in normal vocal fold tissue where significant vibratory dysfunction occurs. The post-operative histological changes are correlated with the vocal fold vibratory patterns. The miniswine serve as the experimental model because of their histologic similarity to human larynges. Modern laryngeal stroboscopy, photography, and voice recording equipment are utilized to identify the critical depth of vocal fold injury causing significant disruption of the vocal fold vibration.

Materials and Methods

Twelve adult, male, castrated miniswine were used in the study. The miniswine were obtained from a USDA approved source and

guaranteed for 30 days. Pre-operatively they were sedated and given Dicloxacillin 500 mg. They were then intubated and placed on Isoflurane gaseous anesthesia with assisted ventilation. Monitoring was accomplished via end tidal CO₂, pulse oximetry, ECG, and rectal core temperature. Suspension microlaryngoscopy utilizing a Zeiss operating microscope confirmed normal laryngeal anatomy.

The first surgical procedure on the right true vocal fold utilized microsurgical instruments. The surgical incision and/or excision was created in the mid-portion of the right fold and measured approximately 10 mm in length. The depth of injury varied. Group A consisted of three miniswine (numbered 1,2,3), in which the right true vocal fold was incised through the epithelium and lamina propria. Group B consisted of three miniswine (numbered 4,5,6), in which the epithelium and lamina propria were excised to an approximate depth of 1 millimeter (mm). Group C consisted of three miniswine (numbered 7,8,9) in which the excision was carried into the vocalis muscle to an approximate depth between 1-2 mm. Group D consisted of three miniswine (numbered 10,11,12), in which the excision was carried deep into the vocalis muscle to an approximate depth greater than three mm. Figure 1. shows the extent of injury to the right vocal folds. The left true vocal fold was used as a control.

The vocal folds were videotaped using a 0 degree Nagashima telescope attached to a Nagashima Model LS-3A laryngo-stroboscope. A Toshiba TV camera and a Sony Video 8 recorder/player were used to film the vocal folds pre-operatively and post-operatively during the first procedure. The tissue removed from the right vocal fold was sent to pathology for verification of the depth of excision. Post-operatively the animals were recovered in a climate controlled CCU until awake and then were returned to the pen with feed and water. The miniswine were allowed to heal for four to six weeks. Post-operative care included Dicloxacillin 500 mg twice a day for one day. No significant post-operative complications occurred.

After the healing phase, a second procedure was performed using the method described by Rubin²⁴ and Berke²⁹ for dogs, utilizing the general endotracheal anesthesia and direct microlaryngoscopy as previously described. A tracheostomy was created at the thoracic inlet for ventilation. A vertical midline incision was made in the neck to allow access to the larynx and trachea. Lateral retraction of the strap muscles and sternocleidomastoid muscles provided the exposure necessary to identify the recurrent laryngeal nerves lying superficially in the fascia and medial to the carotid sheath. A 1 cm portion of each recurrent laryngeal nerve was carefully dissected inferior to the larynx to allow application of bipolar, insulated Grass electrodes. The external branch of the superior laryngeal nerve was not isolated. Instead, Grass electrodes were

directly inserted into the cricothyroid muscle. A distal tracheotomy was performed and a cuffed Shiley tracheostomy tube inserted to permit ventilation or spontaneous breathing. The more proximal tracheotomy allowed insertion of a cuffed tracheostomy tube 2 cm below the vocal folds through which oxygen flowed in a cephalad direction at a rate of 4 liter per minute. The oxygen flow was humidified and maintained at a constant temperature of 37 degrees centigrade by a 3-M Bird Products Humidifier Controller. Vibrating vocal folds that appeared dry after prolonged stimulation were moistened with normal saline. A microphone was suspended 5 cm above the vocal folds and connected to a Nagashima laryngostroboscope Model LS-3A for frequency control of the stroboscope flashes. One multi-channel nerve/muscle stimulator was used (JOANCO Medical Electronics, LTD Model 4). The recurrent laryngeal nerves stimulus voltage ranged from 0.5V-0.9V and the muscle stimulus voltage ranged from 3.0V-6.0V. The stimulus frequency was 60 Hz with a pulse duration of 0.1-0.2 ms. This was sufficient to produce vocal fold lengthening and adduction.

Direct laryngostroboscopy was performed using a 0 degree Nagashima laryngoscope with integrated fiber optic light cable attached to a Nagashima laryngostroboscope Model LS-3A. The laryngoscope was inserted through the thyrohyoid membrane and positioned at the level of the false folds while viewing supraglottal segments. Subglottic stroboscopy was performed by

inserting the 70 degree Nagashima laryngoscope through a small incision in the cricothyroid space. The stroboscope was controlled by automatic synchronization with the miniswine phonation. A video camera (Toshiba Model 5N-3) was used to record stroboscopy images onto a video 8 recorder/player (Sony).

Simulation of normal phonation consisted of stimulating both recurrent laryngeal nerves and the cricothyroid muscle. The large intercartilaginous space of the dorsal portion of the miniswine larynx caused significant escape of air requiring closure of the space by suturing with a figure-of-eight 4-0 nylon suture. This step allowed increased airflow through the area of the vocal folds.

The electroglottograph (EGG) provides a means of obtaining information about vocal fold function that is non-invasive. The EGG waveform corresponds to the surface contact^{10,11} area of the vocal folds and is measured by placing a pair of plate electrodes near the thyroid cartilage. Electrical impedance changes across the larynx are obtained by the EGG. The impedance is least when the vocal folds are in full contact, and impedance increases as the vocal folds open. These impedance measurements are captured by computer software.

The EGG tracings were obtained by positioning the electrodes of the Laryngograph¹² (Model 6091 Kay Elemetrics Corporation) on

either side of the miniswine larynx. The signals were directed into an AT-compatible computer equipped with a plug-in card that provided a real-time waveform capture and display system (Waveform Display System, Model 6091). This software allowed data to be stored for documentation. Figure 2. shows the arrangement of equipment for the voice laboratory and Figure 3. shows the recording equipment as arranged for the miniswine.

Subglottic pressure was measured by a 14 gauge catheter inserted through the interarytenoid space and threaded so the tip of the catheter was below the vocal folds. This catheter connected to a manometer that provided subglottic pressure measures. The subglottic pressure was maintained between 4 and 8 inches of H₂O with vocal fold closure, and between 1 and 3 inches of H₂O with vocal fold vibration.

Figure 4. shows sequential photographs of the miniswine vocal fold traveling wave effect. These photographs were obtained with a modified Pentax SRL camera with a 49 mm macro lens, ASA 100 color film and an exposure time of 1/60 second. The photographs of stop frame images were taken after the video 8 tape was processed through a Zenith 248 computer equipped with a Targa Plus board (TrueVision, Inc.) & TIPS Software (TrueVision, Inc.). Slides were made on Montage FRI (Presentation Technologies, Inc.) via a RASCOL board.

As each study was concluded, the miniswine received euthanasia via intravenous injection of sodium pentobarbital solution 0.3 ml/lb body weight intravenously. The miniswine larynx was excised and sent to pathology. All surgical specimens were fixed in 10% buffered formalin prior to histologic examination. After fixation, each healed larynx was bisected in the midsagittal plane. Both membranous portions of the true vocal folds of each larynx were sampled at their mid-points. The excised sample contained the full thickness of the laryngeal wall. The sections were embedded in paraffin and sectioned at 6 microns. The samples were stained with hematoxylin and eosin, Massons Trichrome and Verhoff's elastic stain. The thickness of the mucosa at the free edge of the membranous portion of the true vocal cord was measured with a micrometer. The amount of elastic and collagenous tissue was quantified as follows: decreased, no change or increased. The pathology evaluation was performed as a blind study by a board certified pathologist.

Results

The stroboscopy findings were analyzed according to the parameters established by Hirano¹⁶. The vocal fold parameters used for this study are: 1) periodicity, 2) glottic closure, 3) amplitude of motion, 4) stiffness, 5) symmetry, and 6) mucosal wave. A brief explanation of these parameters is appropriate.

Periodicity is the regularity of successive cycles of vocal fold vibration and is rated as regular, irregular, or inconsistent. Glottic closure is determined by the extent to which the vocal folds approximate during the closed phase of the vibratory cycle and is rated as complete or incomplete. The amplitude of vocal fold motion is defined as the extent of horizontal excursion of the vocal folds during their movement, and each vocal fold is rated independently. Stiffness indicates immobility of the vocal fold and is rated as an increase or decrease. Symmetry is the degree to which the vocal folds provide mirror images of each other and is rated as symmetric or asymmetric. The mucosal wave is the extent of movement of the vocal fold mucosal surface and is rated as an increase or decrease. All observations were made after the four to six week healing phase of the injured right true vocal fold.

The vocal fold periodicity was regular after simple excision of the cover, but irregular and inconsistent after injury to the vocalis muscle. Glottic closure was complete in all of the miniswine vocal folds except in one instance. During the healing phase in miniswine #8 in which the cover and superficial layers of the vocalis were removed, pedunculated granulation tissue developed on the posterior one third edge of the right true vocal fold (Fig. 5). This tissue would intermittently swing between the vibrating vocal folds and cause inconsistent and incomplete closure. Amplitude of motion of the unoperated left true vocal fold and the

operated right true vocal fold was equal until injury occurred to the vocalis muscle. Increased stiffness was not observed until the deep layers of the vocalis muscle were violated. Symmetry was intact until the cover of the folds was excised. Once the cover and deeper layers were excised, asymmetry between the two folds occurred. The mucosal wave decreased after the vocalis muscle was injured. Table II summarizes these findings.

The parameters obtained by the EGG were: fundamental frequency (F_0); and, open quotient (OQ). Frequently, two frequencies were concomitant. For example, for miniswine #12 during post-operative testing, the first frequency of 54.4 Hz resulted from vibration of both the false vocal folds and true vocal folds. The second frequency of 341.3 Hz represented the F_0 of the true vocal folds. Observation of the true vocal folds by the stroboscope required separation of the false vocal folds using a short length of a rubber catheter attached to the tip of the stroboscope. Otherwise, the false vocal folds obscured the view. The F_0 of the true vocal folds remained relatively constant despite the progressive injury to the right true vocal fold (Table II). The mean value of the F_0 of the miniswine true vocal folds was 318.0 Hz.

The OQ is the percentage of time that the true vocal fold remains open as compared to the entire true vocal fold cycle. Figure 6. shows the OQ baseline set at 45% of the peak-to-peak EGG

amplitude of each wave. The OQ increased from 45% to 69% as the severity of injury to the right true vocal fold increased (Fig. 6).

The histological results were described by comparing the left true vocal fold that served as a control and the right true vocal fold that was surgically altered. In the control study, the left true vocal fold and the subglottic region exhibit an angular configuration (Fig. 7A). This configuration differs from the study by Kurita et al²³ in which the free edge is rounded rather than angulated in shape. The epithelial cover of the membranous portion of the control true vocal fold consists of nonkeratinizing stratified squamous epithelium overlying a bilayered lamina propria composed of both collagenous and elastic tissue with the former predominating. At the free edge, the epithelial layer is slightly attenuated in comparison to adjacent epithelium of the vestibule and subglottic area. The boundary between stromal layers is indistinct. The stromal elements of the superficial layer are arranged in a somewhat haphazard fashion whereas their counterparts in the deep layer possess increased density and generally show a basketweave configuration as they approach the vocalis muscle. A conus elasticus is absent. In the region of the vestibule, scattered mixed mucous and serous glands are exhibited adjacent to the deep layer or within the superficial layer of vocalis muscle. A second collection of seromucinous glands is present in the

subglottic region. The vocalis muscle is an intact muscle extending above and below the vestibule with closer approximation to the true than false vocal fold. The boundaries of the vocalis muscle and the deep layer of the lamina propria are distinct.

In the surgically altered right true vocal fold several noticeable changes were observed: the free edge changed from an angulated to a rounded configuration (Fig. 7B); the thickness of the cover decreased in the majority of the specimens; and, the collagen content in all specimens increased (Table II) while the elastin content was variable. The average mucosal thickness of the cover of the preoperative true vocal fold was 0.95 mm. In the surgical groups the thickness decreased to an average of 0.52 mm. The change in thickness was related to changes in the lamina propria rather than the epithelium. Groups C and D (miniswine #7 thru #12) showed vocalis muscle elements entrapped in the reparative process involving the lamina propria (Fig. 7B). These miniswine were exposed to biopsies of the cover and vocalis muscle. The degree of scarring was greater in these groups. Vocal fold morphology remained intact until the deeper layers of the vocalis muscle were excised and then a soft tissue defect was noticeable along the edge of the right true vocal fold (Table II). No major disruption or loss of the seromucinous glands occurred in the true vocal folds of the test subjects because the location of the glands near the apex of the vestibule, were away from the biopsy site.

Discussion

Miniswine were selected for this study for several reasons. Castrated male miniswine (Sus scrofa) rarely attain weights greater than 120 pounds and can be handled by operating room personnel. The larynx size is comparable to the human larynx, and the miniswine's short neck allows microsurgical instrumentation similar to that used in humans. Extended instrument length often required for dogs is not needed for the miniswine. The miniswine is readily available and less costly. Histologically, the lamina propria of the miniswine vocal fold has indistinct boundaries, only two layers, and no vocal ligament or conus elasticus. These are similar findings to the vocal fold of a child¹². The density of collagenous fibers in the miniswine lamina propria increases as the vocalis muscle is approached, which is another unique similarity to the human vocal fold. The miniswine vocal folds appear to be better suited for study when compared to the dog vocal fold which differs significantly. In addition, the domesticated dog and other companion animal species are currently more difficult to obtain due to successful political efforts by animal rights organizations. Despite the advantage that the miniswine model offers, it is unlikely that the vibratory characteristics of any animal are exactly comparable to that of humans. This caveat must be kept in mind when drawing conclusions from the study.

The study of vocal fold physiology has primarily focused on normal function and has been performed mostly by European and Japanese researchers in the last decade. Stroboscopy technology is now available to clinicians and is presently opening a new frontier to the otolaryngologist. This miniswine study focuses on vocal fold function after progressive surgical injury to the fold. Generations of otolaryngologists have performed laryngeal surgery on patients with vocal fold pathology, yet few guidelines have been established regarding the limits of resectability before voice quality is compromised. Traditional "stripping" procedures have been shown to produce changes in fundamental frequency^{33,34} but in most instances a disregard prevailed for the quality of the post-operative voice. The introduction of microsurgical techniques utilizing the operating microscope and advanced laser technology was a significant step forward in the 1970's³⁵. Microlaryngeal surgery provided the otolaryngologist an opportunity to selectively remove vocal fold pathology and still preserve the maximum amount of normal tissue with minimal disruption to vocal fold function. This study helps define the limits of dissection utilizing current technology to preserve voice quality.

Videostroboscopy of the vocal fold function after progressive injury and healing of the right fold produced significant findings. Loss of symmetry of the vocal folds when comparing the injured right fold to the uninjured left fold was the earliest observed indication of vibratory dysfunction. This result was produced when

the cover of the vocal folds was excised. Consistent changes in periodicity, amplitude and the mucosal wave occurred after deeper injury to the cover and the superficial layers of the vocalis muscles. Only the deepest injury to the vocal fold caused increased stiffness. Glottic closure was the least reliable parameter. Despite the depth of injury to the vocal fold, glottic closure was achieved in all instances. This observation is probably due to the artificial situation created in the laboratory in which increased electrical stimulation of both nerve and muscle eventually resulted in closure. Obvious changes in vocal fold morphology occurred only after the deepest injury of the vocalis muscle. The miniswine vocal fold healed quickly and tissue defects were observed only after excisional biopsy deep into the vocalis muscle (Fig. 8).

The stroboscopic observations consistently correlated with the histologic findings. As the injury to the vocal fold progressed from the cover to the deeper layers of the vocalis muscle, chaotic vibratory patterns increased. Injury to the cover caused minimal changes, but injury to the vocalis muscle caused significant problems. Non-vibratory segments often documented by stroboscopy in the injured vocal folds of humans were not evident in the miniswine. This finding may be due to the absence of a conus elasticus in the miniswine. Despite severe injury to the miniswine vocal fold, vibration appears to persist because the layers of the lamina propria are loose and indistinct.

Significant scar formation failed to materialize. In all the injured vocal folds of the miniswine, an increase in the collagen content was observed. Because the miniswine vocal fold has less collagen than the human vocal fold less scar formation occurs. Persistent granulation tissue during the healing phase in microswine #8 accounted for a single discrepancy in the study.

Interpretation of the EGG results showed no significant deviation of the F_0 of the true vocal fold even when the cover and body were deeply injured. Hirano²¹ classified the extent of vocal fold tissue removed in 16 patients operated upon with T_1 glottic carcinoma, and he also observed no marked difference in the F_0 range of phonation. A larger study by Hirano²² again showed no increase in F_0 for vocal fold surgery for nodules, hyperplasia, and carcinoma. This finding was probably due to the healing ability of the vocal folds. At the time of phonatory function testing, all miniswine had a vocal fold-like structure on the operated side, even after removal of the deep layers of the vocalis muscle. Changes in mass and stiffness did not appear to be sufficient enough to create significant changes in F_0 .

The increase in OQ was significant because it provided a more sensitive measure. After injury and healing to the right true vocal fold, the percentage of time the vocal fold remained open increased as the depth of injury increased. This is probably due

to the scar formation causing an increase in stiffness. Slight bowing of the healed vocal fold on the injured side could also contribute to a larger OQ. Prolonged or incomplete glottic closure was not readily observed during stroboscopy. The EGG which provided a more sensitive measure complimented the stroboscopy findings that rely heavily on the observer's visual interpretation.

As in stroboscopy, the histological findings compared consistently with the EGG results. The OQ increased as the injury to the vocal fold progressed to the deeper layers of the vocalis muscle. The thickness of the epithelial cover remained the same after healing but the thickness of the lamina propria was diminished by half. This decrease in thickness, along with a rounding effect of the free edge of the vocal fold, probably accounts for the increase of the opening phase of the vibratory cycle. The electroglottograph OQ measured this effectively and provided the earliest indication of vibratory dysfunction.

The clinical implication of this study reinforces the knowledge that progressively deeper injury to the vocal fold will result in more abnormal vibratory functions as measured by stroboscopy and EGG. These results correlate with histological findings. Injury superficial to the junction of the lamina propria and the vocalis muscle cause subtle change. Injury at the junction of the lamina propria and deeper into the vocalis muscle cause

significant and consistent disruption of the vibratory pattern of the vocal fold. This unique surgical model demonstrates for the laryngeal surgeon the critical depth of injury to the vocal folds where the functional voice is adversely affected.

Conclusions

1. The miniswine vocal fold is a useful model in which to study vibratory function.
2. The open quotient of the EGG detected abnormal vibratory patterns earlier than stroboscopy.
3. Stroboscopy and EGG results correlated with the histological findings; i.e., the deeper the wound the more abnormal the vibratory pattern.
4. Injury at the junction of the lamina propria and vocalis muscle caused consistently abnormal vibratory function.
5. Because of the similarity between the vocal folds of both miniswine and humans, injury beyond the junction of the lamina propria and the vocalis muscle will likely cause unacceptable phonation.

TABLE I.

Comparison of Vocal Folds

	HUMAN	MINI-SWINE	DOG
1. VENTRICLE	YES	YES	YES
2. CONUS ELASTICUS	YES	NO	YES
3. MEMBRANOUS LENGTH (A)	15MM	18MM	15MM
4. MUCOSAL THICKNESS (B)	1.1MM	0.9MM	3.0MM
5. RATIO A:B	14:1	20:1	5:1
6. LAYERS	3	2	2

TABLE II
Miniswine Vocal Fold Findings

	Periodicity	Glottic Closure	Amplitude	Stiffness	Symmetry	Mucosal Wave	Fundamental Frequency (F ₀)	Open Quotient (OQ)	Collagen Content	Morphology
Group A	Regular	Complete	Equal	Normal	Symmetric	Normal	Stable	Stable	Increased	Normal
Group B	Regular	Complete	Equal	Normal	Asymmetric	Normal	Stable	Increased	Increased	Normal
Group C	Irregular	Complete	Right decreased	Increased	Asymmetric	Decreased	Stable	Increased	Increased	Irregular free edge
Group D	Irregular	Complete	Right decreased	Increased	Asymmetric	Decreased	Stable	Increased	Increased	Tissue defect

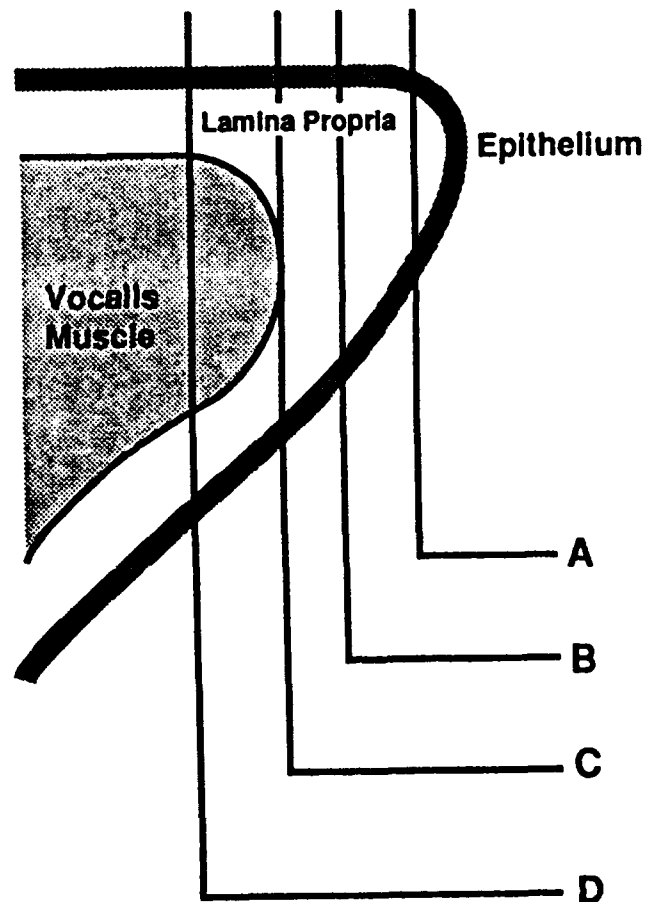


FIG. 1. THIS CLASSIFICATION DEPICTS THE EXTENT OF THE VOCAL FOLD TISSUE INCISED OR EXCISED:

GROUP A...INCISION THROUGH THE EPITHELIUM
AND LAMINA PROPRIA

GROUP B...EXCISION THROUGH THE EPITHELIUM
AND LAMINA PROPRIA

GROUP C...EXCISION TO THE LEVEL OF THE
VOCALIS MUSCLE

GROUP D...EXCISION DEEP INTO THE VOCALIS
MUSCLE

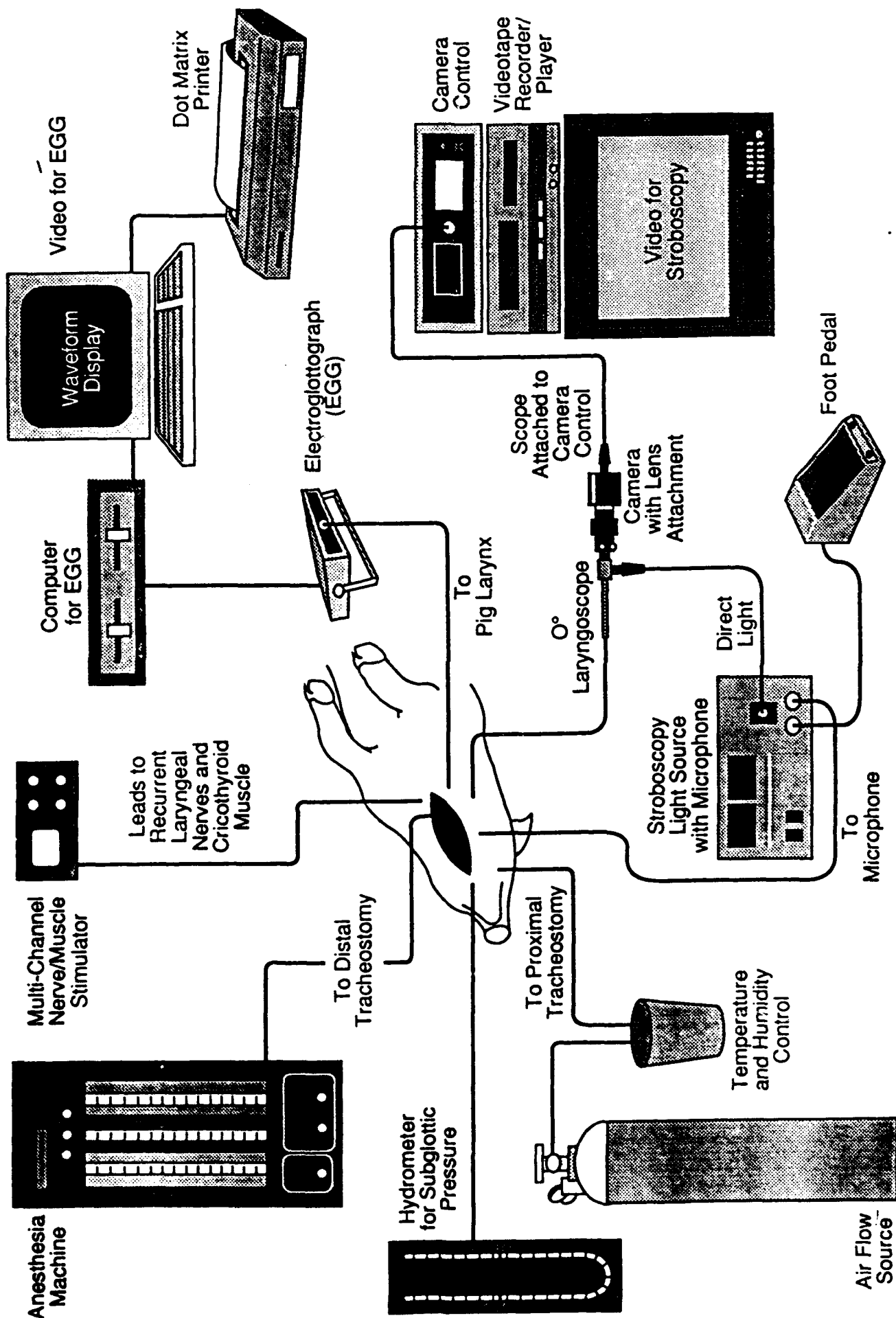


FIG. 2. ARRANGEMENT OF EQUIPMENT FOR THE VOICE LABORATORY

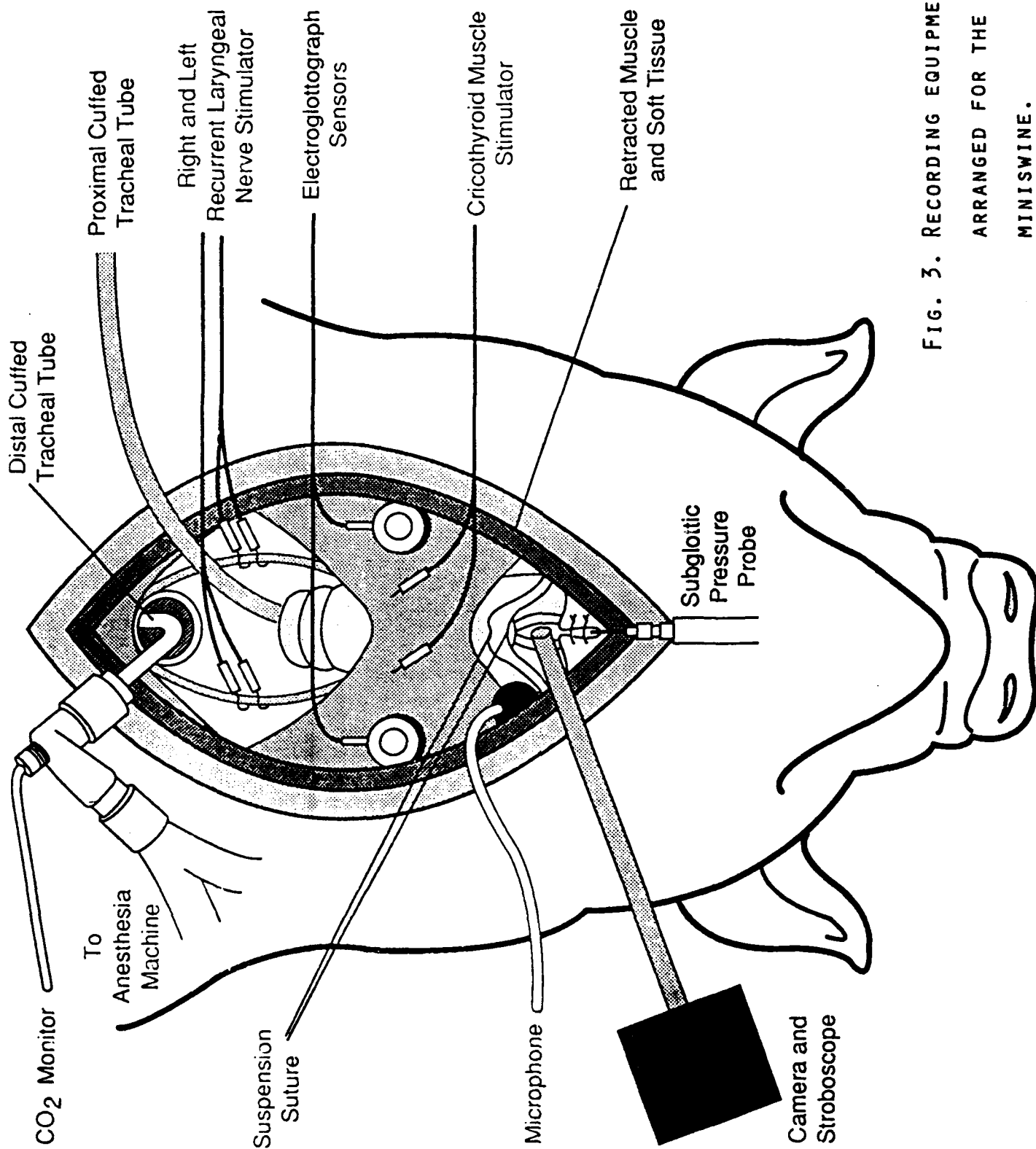


FIG. 3. RECORDING EQUIPMENT
ARRANGED FOR THE
MINISWINE.

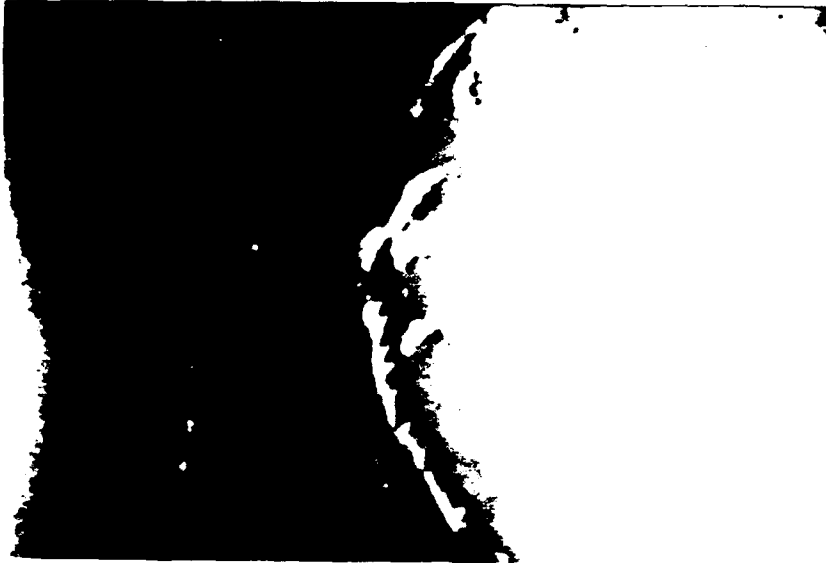




FIG. 4. SEQUENTIAL PHOTOS OF THE
MINISWINE VOCAL FOLD
TRAVELING WAVE EFFECT.



FIG.5. PEDUNCULATED GRANULATION
TISSUE OF THE POSTERIOR
ONE-THIRD EDGE OF THE
RIGHT TRUE VOCAL FOLD.

WAVEFORM DISPLAY

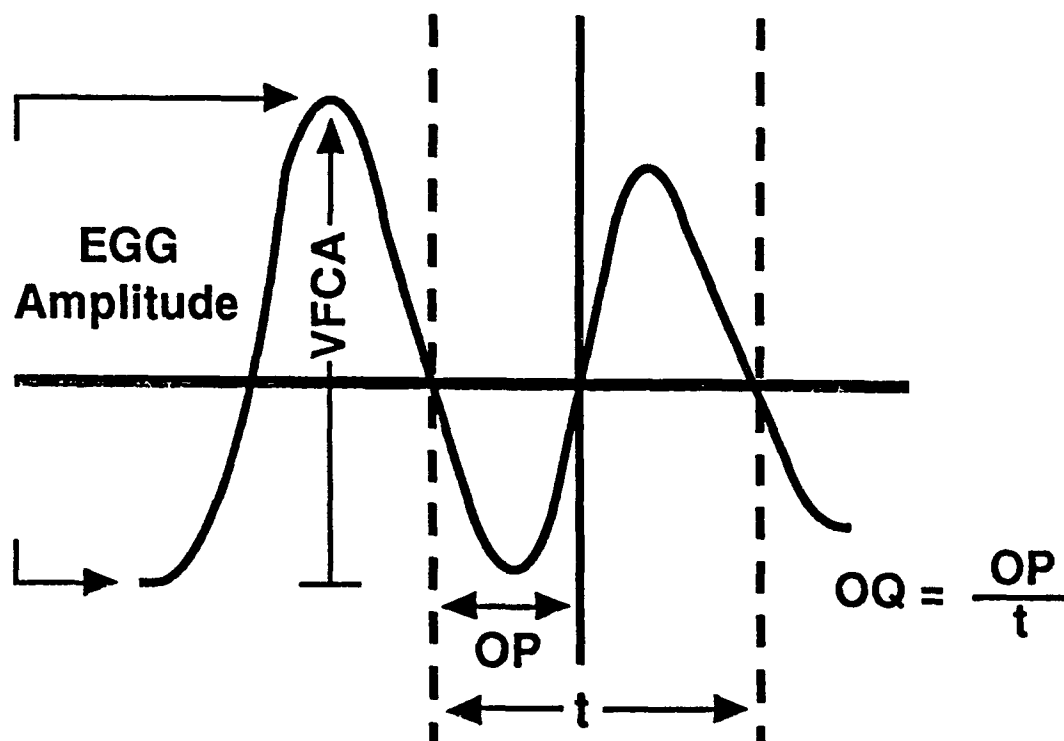


FIG. 6. SCHEMATIC REPRESENTATION OF THE ELECTROGLOTTOGRAPHIC LANDMARKS AND INTERVALS USED TO DERIVE THE OPEN QUOTIENT (OQ). THE HORIZONTAL DASHED LINE REPRESENTS 45% OF THE PEAK-TO-PEAK EGG AMPLITUDE; VFCA = VOCAL FOLD CONTACT AREA; OP = OPEN PHASE; T = TIME FOR COMPLETE CYCLE.

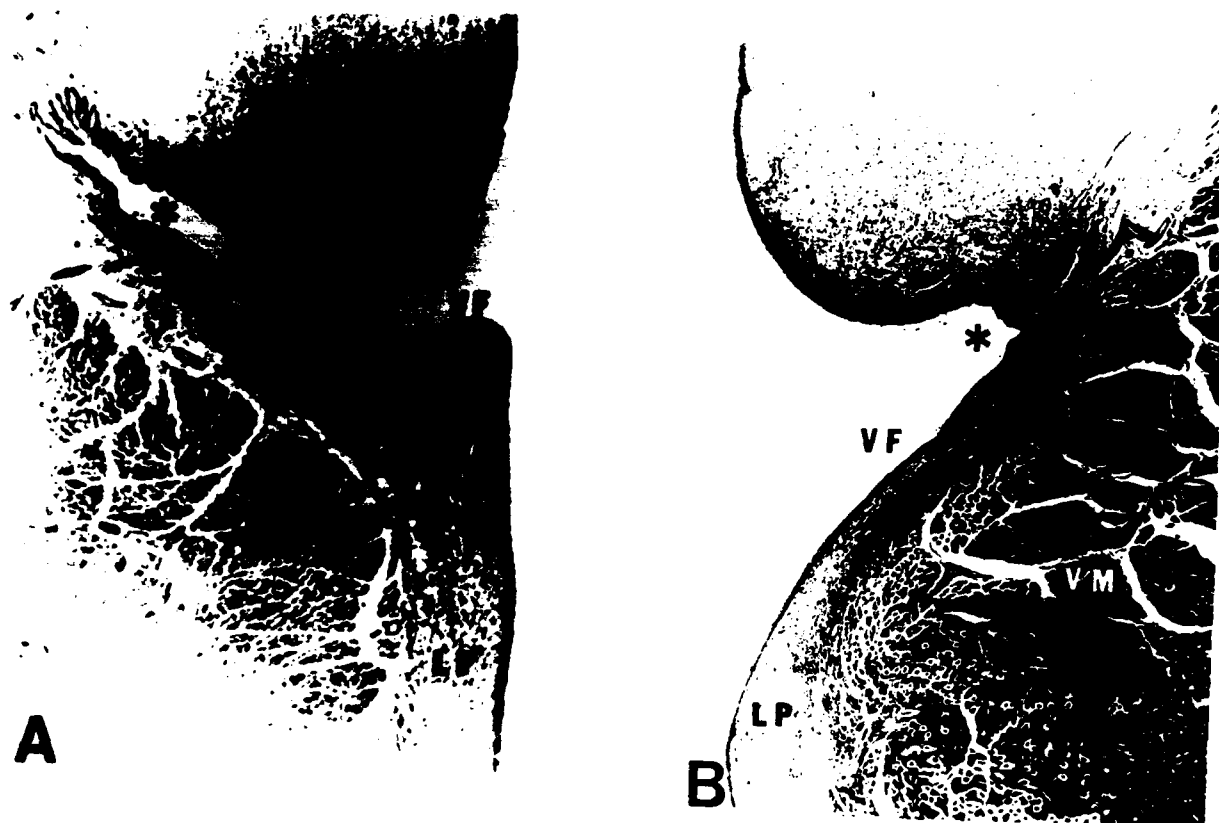


FIG. 7. VIEW A IS A CROSS-SECTION OF THE UNOPERATED LEFT TRUE VOCAL FOLD OF THE MINISWINE (HEMATOXYLINE AND EOSIN AT 20X MAGNIFICATION). VIEW B IS A CROSS-SECTION OF THE INJURED RIGHT TRUE VOCAL FOLD (MASSONS TRICHROME AT 20X MAGNIFICATION). THE ARCHITECTURAL DISTORTION OF THE VOCAL FOLD (VF) FREE EDGE CAN BE COMPARED BETWEEN THE LEFT AND RIGHT FOLDS. VM, VOCALIS MUSCLE; LP, LAMINA PROPRIA; (*), VESTIBULE.

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